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## Validity and Redescription of *Profundulus balsanus* Ahl, 1935 (Cyprinodontiformes: Profundulidae)

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### Abstract

*Profundulus balsanus* was described by Ahl in 1935 and later placed in synonymy with *P. punctatus*. Recent molecular studies have demonstrated that specimens identified as *P. balsanus* belong to a distinct taxon. Herein we used a combination of morphometric, meristic, coloration and osteological data in order to demonstrate the distinctiveness of *P. balsanus*. Additionally, we used molecular data to reconstruct a phylogeny to place *P. balsanus* in a systematic context. *Profundulus balsanus* belongs to the *punctatus* clade within the genus *Profundulus*. It can be distinguished from *P. oaxacae* based on scale count in the lateral line and from *P. punctatus* based on differences in coloration in the anal fin. We provide a taxonomic key to all Mexican species of *Profundulus*.

**Key words:** meristic, fish systematics, taxonomy, southern Mexico, synonyms

### Introduction

*Profundulus balsanus* (Figs. 1 & 2A–B) was originally described by the German ichthyologist Ernst Ahl 1935, based on twelve specimens collected in the Malinaltepec River, a tributary of the Papagayo River (Balsas River in original description), in a locality close to the town of Malinaltepec, Guerrero, Mexico (Fig. 3). The brief description provided data for minimal morphometric (e.g., body-depth to body-length, snout to head-length, interorbital-width and head-length ratios) and meristic (e.g., dorsal and anal-fin ray counts as well as the number of scales along the lateral line) characters. He additionally described the coloration in alcohol and reported a maximum total length of 40 mm based on the studied specimens.

For the next 20 years *P. balsanus* remained a valid species until Miller (1955) placed it in synonymy with *P. punctatus* in a systematic review of the genus *Profundulus*. Miller's taxonomic revision of *Profundulus* is by far the most complete and exhaustive morphological treatment of the group. However, despite the outstanding sample sizes and geographic coverage in Miller's revision, he had available only two poorly preserved specimens from the *P. balsanus* type series (Miller 1950; Miller 1955; UMMZ 157291 [ex ZMB]). At the end of this study Miller was uncertain about two issues related to taxonomy of *P. balsanus*. First, he was not sure if the *P. balsanus* specimens actually came from the Balsas River system; second, he affirmed that because the *P. balsanus* samples he used were poorly preserved, he could not conclusively determine if they were a valid taxon, and therefore suggested that *P. balsanus* be temporarily placed as a synonym of *P. punctatus* (Miller 1955). Miller *et al.* (2005) continued to recognize *P. balsanus* as a synonym of *P. punctatus*.

The taxonomic status of *P. balsanus* as a synonym of *P. punctatus* was never questioned until a recent study that assessed genetic divergence and biogeography of species in the subgenus *Profundulus*. Doadrio *et al.* (1999) concluded that *P. punctatus* is a complex of genetically distinct species that includes *P. punctatus*, *P. guatemalensis*, and *P. oaxacae*, as well as *Profundulus* sp.1, *Profundulus* sp. 2, and *P. balsanus*. They also showed that *P. balsanus* possesses two unique alleles that are not shared with other members of the subgenus *Profundulus* (Doadrio *et al.* 1999). In a later molecular study aimed at assessing the phylogeography and evolutionary history of the family Profundulidae, Morcillo *et al.* (2016), using three mitochondrial (ATPs 8-6 and ND2) genes and one nuclear (S7) gene, recovered *P. balsanus* as a distinct lineage with average mitochondrial divergence (*p* distances) between *P. balsanus* and its congeners ranging from 3.4 to 10.6%. They also suggested that *P. balsanus* should be recognized as a valid taxon based on its phylogenetic distinctiveness, with a distributional range that includes lowland and highland areas of the states of Oaxaca and Guerrero, Mexico. Furthermore, Morcillo *et al.* (2016) suggested that the two subgenera (*Tlaloc* and *Profundulus*) should be erected to genus level.

Herein we redescribe *P. balsanus* based on a combination of standard morphometric and meristic data. We explored variability in meristic and morphometric characters using Principal Component Analysis (PCA) and further assessed genetic distinctiveness of *P. balsanus* by constructing a molecular phylogenetic hypothesis based on two mitochondrial genes and increased distributional sampling of specimens.

## Materials and methods

**Study site and fishing gear.** From April 21 to May 1, 2015, 23 localities were sampled in the Mexican states of Chiapas, Oaxaca and Guerrero; including the Malinaltepec River near the type locality of *P. balsanus*, in Malinaltepec, Guerrero (Figs. 2D & 3). Fishes were captured using a combination of fishing gear that included seines, cast-nets and electrofishing. After capture, fishes were euthanized with an dose of MS222. In the field, a fin clip was excised from specimens and preserved in 95% ethanol. Fishes were then fixed in a solution of 10% formalin for at least 72 hours, then rinsed for 24 hours in tap water and immediately transferred to a solution of 75% ethanol for final preservation. All samples were deposited at the fish collection of the Universidad de Ciencias y Artes de Chiapas (MZ-P-UNICACH). Material examined is provided in the Appendix of this paper.

**Measurements and counts.** Unless noted, all measurements and counts were performed following Miller (1948), including: Standard Length (SL), Head Length (HL), Predorsal Length (PDL), Prepelvic Length (PPL), Anal Origin to Caudal Base (AOCB), Body-Greatest Depth (BGD), Body-Greatest Width (BGW), Caudle Peduncle-Length (CPL), Caudle Peduncle-Least Depth (CPLD), Dorsal Fin-Basal Length, (DFBL), Anal Fin-Basal Length (AFBL), Head Depth (HD), Head Width (HW), Interorbital-Least Bony Width (IOLBW), Orbit Length (OL), Snout Length (SNL) and Upper Jaw Length (UJL). All measurements were taken to the nearest 0.01 mm with digital calipers. For analysis, all measurements localized in the head were standardized to percentage of HL; all remaining measurements were standardized to percentage of SL.

Meristic counts included anal-fin rays (AR), dorsal-fin rays (DR), principal caudal-fin rays (CR), principal pectoral-fin rays (PR), principal pelvic-fin rays (PER), lateral-line scales (LSC), pre-dorsal scales (PSC), scales count around peduncle (SCAP) and scale count around the body (SCAB). For the SCAB we started to count one scale anterior to the origin of the dorsal fin. For the DR and AR we counted the last two rays as one as suggested by Miller (1948), since in Cyprinodontiformes the last ray bifurcates inside the body (Miller, 1948). The CR count was performed by counting all principal rays (i.e., bifurcate) plus two (one unbranched in each side) as suggested by Miller (1948).

To graphically represent the variability of meristic and morphometric (excluding SL) data between *P. balsanus*, *P. punctatus* and *P. oaxacae* in multivariate space, we performed a Principal Components Analysis (PCA). PCA was implemented in the R 3.0.2. statistical software (R Development Core Team 2013) package ‘vegan’ (Oksanen *et al.* 2012).

**Molecular phylogeny.** Whole genomic DNA was extracted from tissues using the Qiagen DNeasy Tissue Kit (Qiagen Inc., Valencia, CA). Two mitochondrial genes were sequenced; cytochrome *b* (cyt *b*) and cytochrome oxidase subunit 1 (COI). The cyt *b* gene was amplified using primers GluDGL and H16460 (Palumbi 1996 and Perdices *et al.* 2002 respectively), as well as PCR protocols from Perdices *et al.* 2002. The COI gene was amplified using the primers FISH-BCL and Fish-BCH (Baldwin *et al.* 2009) and the PCR protocols presented in Baldwin *et*

*al.* (2009). PCR products were sequenced at the Smithsonian Tropical Research Institute in Naos, Panama City, Panama. Chromatographs were checked by eye for ambiguities and manually assembled using Geneious 5.4 (Kearse *et al.* 2012). We used MUSCLE (Edgar 2004) embedded in MEGA6 (Tamura *et al.* 2013) to align sequences for each individual gene. Alignments were inspected by eye for accuracy, and sequences were trimmed at the 3' and 5' ends to minimize missing characters. We calculated between species genetic distances (*p*-distances) in MEGA6 (Tamura *et al.* 2013). The final data matrix consisted of 1,104 bp for *cyt b* and 633 bp for *COI*, for a total of 1737 nucleotides in the concatenated analysis. All newly generated sequences were deposited in GenBank (Table 1). We used jModelTest (Posada 2008) to select the best fitting model of sequence evolution under the corrected Akaike Information Criterion (AICc; Akaike 1973). jModelTest selected TPM2uf+I and TIM2+I+G as the best models for the *cyt b* and *COI*, respectively.

**TABLE 1.** GenBank accession numbers for sequences used in this study.

Species	Cyt-b	COI
<i>Profundulus guatemalensis</i>	AY155568	N/A
<i>Profundulus kreiseri</i>	JQ254934	N/A
<i>Profundulus punctatus</i>	KX611591	KX611585
<i>Profundulus oaxacae</i>	JQ254933	KX611584
<i>Profundulus balsanus_1</i>	KX611586	KX611579
<i>Profundulus balsanus_2</i>	KX611587	KX611580
<i>Profundulus balsanus_3</i>	KX611588	KX611581
<i>Profundulus balsanus_4</i>	KX611589	KX611582
<i>Profundulus balsanus_5</i>	KX611590	KX611583
<i>Tlaloc labialis</i>	AY155567	JN028283
<i>Tlaloc candalaria</i>	JQ254931	KX632152
<i>Tlaloc hildebrandi</i>	JQ254932	KX632151
<i>Tlaloc portillorum</i>	JQ254930	N/A
<i>Ilyodon whitei</i>	AF510832	AY356580
<i>Ilyodon furcidens</i>	AF510831	AY356579

The two individual gene datasets were concatenated in Mesquite 2.75 (Maddison & Maddison 2011), and used in Bayesian analyses using MrBayes 3.2 (Ronquist *et al.* 2012). We partitioned the concatenated dataset by locus and assigned the best fitting models selected by jModelTest. Two analyses of one million generations each were performed with four chains (one cold, three heated) sampling every 1000 generations. We used Tracer 1.5 (Rambaut and Drummond 2019) to check the trace files and ensure the chains had reached convergence, and discarded the first 25% of trees as burn-in. A 50% majority rule consensus tree was obtained from post-burn-in trees.

## Results

### Redescription of *Profundulus balsanus* Ahl, 1935

Figs. 1, 2A–B & 4. Table 2

**Common names.** Balsas killifish, popoyote del Balsas.

**Lectotype.** ZMB 21094, Río Balsas system, Malinaltepec, Guerrero, Mexico (see distribution section for locality corrections).

**Diagnosis.** *Profundulus balsanus* (Figs. 1 & 2A–B) is phylogenetically recovered as a member of the genus *Profundulus* (Fig. 4) and differs from all species of *Tlaloc* (*sensu* Morcillo *et al.* 2016) by the presence of a humeral spot, a preorbital bone covered with well-developed scales and more than half of the anal fin covered with scales (Miller 1955, Matamoros & Schaefer 2010, Matamoros *et al.* 2012, Morcillo *et al.* 2016). *Profundulus balsanus*

can be distinguished from other members of the genus *Profundulus* by possession of 33 scales along the lateral line versus 30 in *P. oaxacae*. *Profundulus balsanus* can be distinguished from *P. punctatus* by the presence of a transparent anal fin without any marks or dark lines extending across the entirety of the fin (Fig. 5A1–A4), versus presence of a dark transverse band in the anal fin of *P. punctatus* located distally on the anal fin but without reaching the distal edge of the fin (Fig. 5B1–B4). Furthermore, *P. punctatus* has a premaxilla with an anterior alveolar process (AALV) that possesses a straight to slightly concave anterior margin, ending ventrally with a slightly round process (Fig. 6), versus *P. balsanus* that possesses a premaxilla with an AALV with a rounded embayed anterior margin that ends in a relatively squared process ventrally (Fig. 6). In *P. punctatus* the posterior margin of the alveolar process (PALV) is concave (Fig. 6) with a wide tip, whereas in *P. balsanus* the PALV is less concave and elongated and ends a sharp tip. *Profundulus balsanus* is restricted to the Mexican states of Oaxaca and Guerrero, whereas *P. guatemalensis* is endemic to a narrow region of the Guatemalan Pacific Slope and *P. kreiseri* is found in the southernmost distribution of the genus in Central America in Guatemala, El Salvador and Honduras (Fig. 3).



FIGURE 1. Photograph of the lectotype of *Profundulus balsanus* (ZMB 21094), 40 mm SL.

**Description.** Morphometric and meristic data are summarized in Table 2. The largest specimen is 68.33 mm SL. The body of *P. balsanus* is elongate and round, with symmetric dorsal and ventral profiles. The narrowest point along the body is the caudal peduncle, which is less dorsoventrally wide than the height of the head. The vertical line through the origin of the dorsal fin is equal to the origin of the anal fin. The head possesses dorsal scales inserted into the skin. The position of the mouth is terminal, with a protruding lower jaw. The dorsal fin has dark pigmentation at the base of the rays. The caudal fin is rounded and the basal half or more of the fin is scaled. The anal fin is usually transparent but sometimes possesses a diffuse line of pigmentation without forming a band (Fig. 5A1–A4). The humeral spot is faded (Fig. 2A–B). Rays: dorsal: 10–14, usually 12; anal: 13–16, usually 15; caudal 18–20; usually 20; pectoral: 11–17, usually 16; pelvic: always six. Scales: scales on the lateral line are always 33, predorsal scales: 18–24, usually 22; scales around the body: 25–29; usually 29; scales around peduncle of 17–21, usually 21.

**Coloration in life.** Live *P. balsanus* are brownish-orange in color. In the anterior half of the body there is a golden-greenish blotch that extends from the preoperculum and operculum to the anterior insertion of the pectoral fin. Along the midline of the body from the posterior insertion of the pectoral fin to the base of the caudal fin, a diffuse dark stripe is present. The anal and caudal fins in males show a yellowish stripe, a series of diffuse dark spots are present in the dorsal fin.

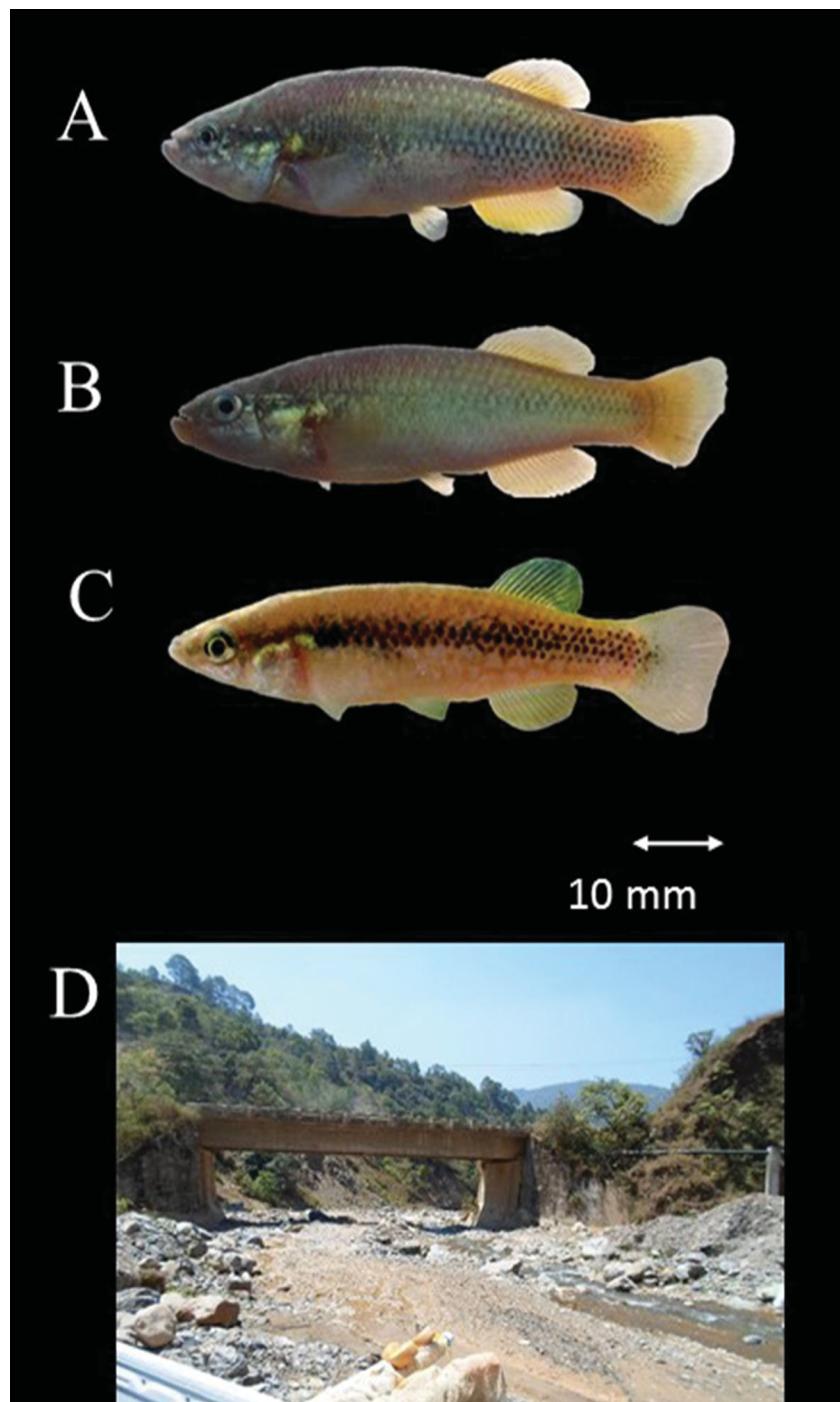
**Coloration in alcohol.** The body is brownish, with a diffuse humeral spot on the back of the operculum; a dark band is present along the midline of the body. The anal fin is yellowish.

**Etymology.** The generic epithet *Profundulus* is derived from the Latin “*profundus*,” meaning “deep”. The specific epithet “*balsanus*” refers to the type locality that was thought to be located in the Balsas River (Ahl 1935); however, the Malinaltepec River where the type locality of *P. balsanus* is located, is a tributary of the Papagayo River, in the Mexican State of Guerrero.

**Distribution.** *Profundulus balsanus* occurs in rivers and streams in the Pacific versant of Mexico in the states of Oaxaca and Guerrero (Fig. 3) at altitudes ranging from 61–1601 meters above sea level. It occurs from the Arroyo Inzcuinatoyac, a tributary to the Papagayo River in the Costa Chica de Guerrero, southward to a locality in the Huatulco River near the city of Santa María Huatulco in the State of Oaxaca; the Huatulco River is a tributary of the Copalita River drainage. Ahl (1935) used the specific epithet “*balsanus*” mistakenly thinking that the locality

in the town of Malinaltepec, Guerrero, belonged to a river that drained to the Balsas River; however, the streams in the town of Malinaltepec all belong to the Papagayo River drainage. Furthermore, no reports of specimens of *Profundulus* collected in the Balsas River exist.

**Phylogenetic distinctiveness.** Results of the Bayesian analysis recovered a consensus tree in which individuals of *P. balsanus* formed a single clade sister to *P. guatemalensis* and *P. kreiseri* (Fig. 4). This result is incongruent with those of Morcillo *et al.* (2016) where they recovered *P. balsanus* sister to a clade formed by *P. punctatus* and *P. oaxacae*. This incongruence could be explained by the additional nuclear data in Morcillo *et al.* (2016); however, *P. balsanus* is recovered as monophyletic in all cases.



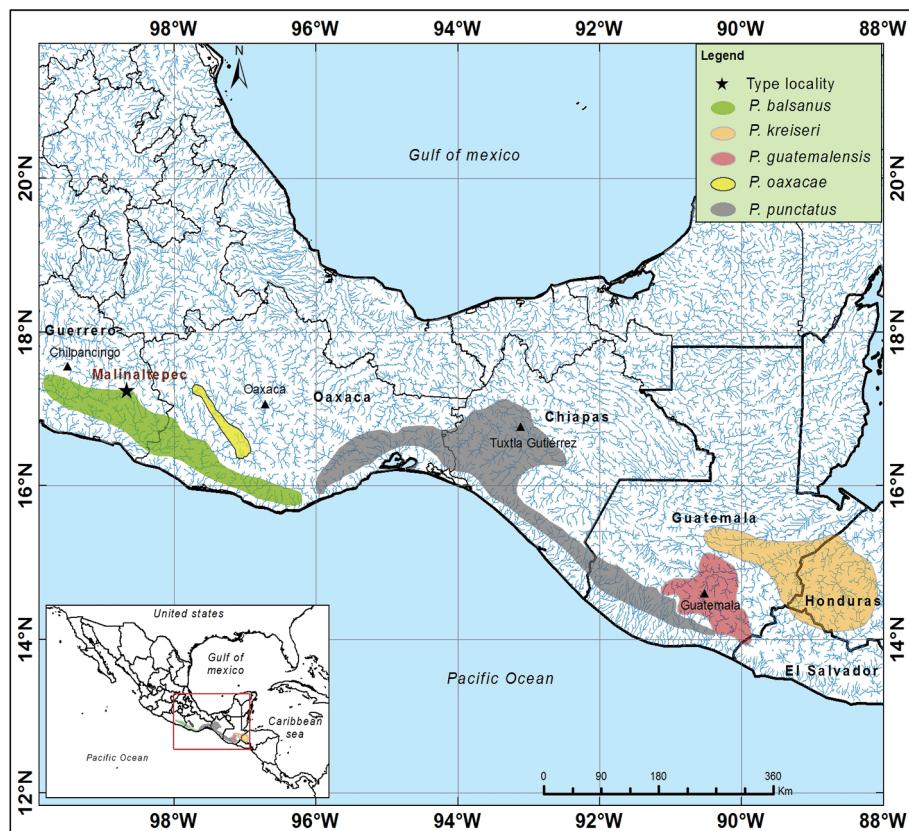
**FIGURE 2.** Photographs of live specimens of *Profundulus balsanus* (A) female, SL = 54.83 mm, and (B) male, SL = 51.30 mm, both 06713-MZ-P-UNICACH from Río Malinaltepec, Malinaltepec, Guerrero. (C) Live specimen of *Profundulus punctatus*, male 06355-MZ-P-UNICACH, from Río Zapata-Puente, Col. Emiliano Zapata, Cacahoatán, Chiapas. (D) Photograph of *P. balsanus* locality (17.236058°N, -98.665656°E) near the type locality in the Río Malinaltepec, municipality of Malinaltepec, Guerrero, Mexico.

**TABLE 2.** Morphometric and meristic data from 22 *Profundulus balsanus* specimens, 36 specimens of *Profundulus oaxacae* and 14 specimens of *Profundulus punctatus*. Measurements are presented as percent of standard length (% SL) or percent of length of the head (% HL). SD = standard deviation, MIN = Minimum, MAX = Maximum.

Profoundulus balsanus				Profoundulus oaxacae				Profoundulus punctatus				
% of Standard Length												
	MEAN	SD	MIN	MAX	MEAN	SD	MIN	MAX	MEAN	SD	MIN	MAX
Head length	26.8	1.6	24.1	30.8	29.3	2.5	26.3	42.1	26.5	2.0	23.6	29.9
Predorsal length	65.5	2.3	61.9	71.7	69.9	5.9	65.5	102.3	67.3	5.9	51.3	77.6
Prepelvic length	51.4	1.9	48.1	55.5	55.1	10.5	47.9	103.9	62.5	7.7	49.2	70.1
Anal origin caudal base	33.4	2.3	29.2	37.1	28.6	2.8	18.0	32.6	32.8	2.1	28.2	36.7
Body-greatest depth	27.8	1.5	25.2	31.0	26.9	2.6	23.5	36.9	26.5	1.8	23.3	29.9
Body-greatest width	17.4	3.3	11.9	22.2	16.5	1.4	14.2	21.7	18.6	2.4	15.8	22.9
Caudle peduncle-length	17.2	1.2	15.5	20.8	14.8	2.2	11.1	22.0	16.5	1.6	13.9	18.5
Caudle peduncle-length depth	13.3	2.4	5.5	15.9	14.6	1.2	11.9	18.9	14.6	0.9	13.7	17.2
Dorsal fin-basal length	15.1	1.6	12.5	19.2	15.6	1.9	12.8	22.3	14.6	1.1	11.6	16.1
Anal fin-basal length	16.1	2.0	13.2	21.0	15.0	2.2	11.7	18.7	15.2	1.2	13.4	17.7
% Head Length												
Head depth	69.3	4.9	56.1	80.6	80.7	5.5	72.1	101.5	70.6	5.8	61.3	79.5
Head width	68.1	5.1	57.9	75.5	64.8	3.0	60.0	72.2	71.3	6.1	60.8	83.8
Interorbital-least bony width	50.3	4.3	38.7	55.9	44.2	1.8	41.5	48.2	52.3	4.4	46.0	59.8
Orbit length	25.0	1.9	21.4	28.9	23.9	1.5	21.1	26.9	26.0	1.8	23.7	28.9
Snouth length	25.3	7.2	15.4	34.9	28.4	2.3	24.0	32.2	31.1	2.2	26.0	35.5
Upper jaw length	16.5	3.0	11.1	23.9	26.5	3.7	19.8	36.1	19.9	5.2	11.3	28.2
	MODA	SD	MIN	MAX	MODA	SD	MIN	MAX	MODA	SD	MIN	MAX
Counts												
Dorsal-fin rays	13	0.71	12	14	12	0.684	11	13	11	0.65	11	13
Anal-fin rays	15	0.316	15	16	14	0.712	13	15	13	0.877	12	15
Principle caudal-fin rays	20	0.632	18	20	20	1.225	17	22	18	1.49	16	22
Principle pelciv-fin rays	16	0.738	15	17	16	0.77	15	18	16	1.051	13	17
Principle pectoral-fin rays	6	0	6	6	6	0	6	6	6	0	6	6
Lateral-line scales	33	0	33	33	30	0.379	29	31	34	1.089	31	34
Predorsal-line scales	24	0.972	21	24	25	1.701	22	29	24	0.392	23	25
Scales count around the body	28	0.966	26	29	27	1.733	21	29	26	0.842	24	26
Scales count around pecuncle	19	0.843	18	21	19	1.273	16	22	18	0.842	17	20

**Multivariate analysis.** The results of the PCA based on morphometric characters showed that the two first PC axes explained 82.5% of the variance, with 62% corresponding to PC1 and 20.5% to PC2 (Fig. 7A). PC1 was related to predorsal length and prepelvic length with a noticeable gradient from negative to positive ends of the axis. *Profoundulus punctatus* and *P. oaxacae* are placed mostly in the negative of PC1, and *P. balsanus* occupied most of the positive side of PC1 in morphospace. For PC2, the most important character was head width, with *P. balsanus* occupying positive areas of PC2.

The PCA based on meristic data (Fig. 7B) explained 59.2% of the variability, of which 35.2% was attributable to PC1 and the remaining 24% to PC2. PC1 was related to AR and DR, whereas PC2 was related to LSC. On average *P. balsanus* showed higher DR and AR counts, whereas the LSC was invariably 33 scales, which averaged higher than *P. oaxacae* and lower than *P. punctatus*.



**FIGURE 3.** Map showing the distribution of *P. balsanus* and other congeners in Mexico and Central America. The star represents type locality of *P. balsanus* in Malinaltepec, Guerrero, Mexico.

## Discussion

*Profundulus balsanus* was placed in synonymy by Miller (1955) and has been treated as a synonym of *P. punctatus* since. However, the distinctiveness of *P. balsanus* has been demonstrated several times based on molecular data (e.g., Doadrio 1999; Morcillo *et al.* 2016 and herein). The differences in relationships between Morcillo *et al.* (2016) and the present study are likely due to the nuclear data in the former study. The goal herein was to use two different markers to further substantiate the phylogenetic distinctiveness of *P. balsanus*. The species is recovered as monophyletic in both analyses. We have morphologically corroborated molecular results. Morphologically we recover a set of diagnostic characters that provide an improved diagnosis and description, including osteological, coloration and meristic characters. Based on these lines of evidence we recognize *P. balsanus* as a valid taxon and provide a redescription of the species and key to all species of Mexican Profundulidae.

## Key to Mexican *Profundulus* (modified from Miller *et al.* 2005)

- 1a. Preorbital nearly covered with well-developed scales; humeral spot present; basal half or more of caudal fin densely scaled; side of adult with longitudinal rows of brown spots; anal fin moderately elongated in mature female only (genus *Profundulus*) ..... 2
- 1b. Preorbital naked or frequently with only 1 or 2 isolated and embedded scales; humeral spot absent; less than basal half of caudal fin densely scaled; no rows of brown spots on side; anal fin of mature female usually markedly elongated (genus *Tlaloc*) ..... 4
- 2a. Scales on the lateral line, typically 30; pattern on the sides, consisting of groomed, faint, vertical marks, whose diameter is the measure of a third of pupil ..... *Profundulus oaxacae*
- 2b. Scales on the lateral line, usually 33 to 34; pattern on the sides, composed of prominent round spots, whose diameter is half of the pupil ..... 3
- 3a. Presence of transverse dark band on anal fin, which extends from the first to the last ray, located near the distal edge of the fin

but without touching its margins; presence of conspicuous humeral spot ..... *Profundulus punctatus*

3b. Absence of transverse dark band on anal fin, rarely with diffuse pigmentation but never forming a band, anal fin usually transparent; presence of very diffuse humeral spot ..... *Profundulus balsanus*

4a. Lower jaw broad, heavy and protruding, extending beyond upper jaw; body uniformly slender, with dark spot at caudal base ..... *Tlaloc hildebrandi*

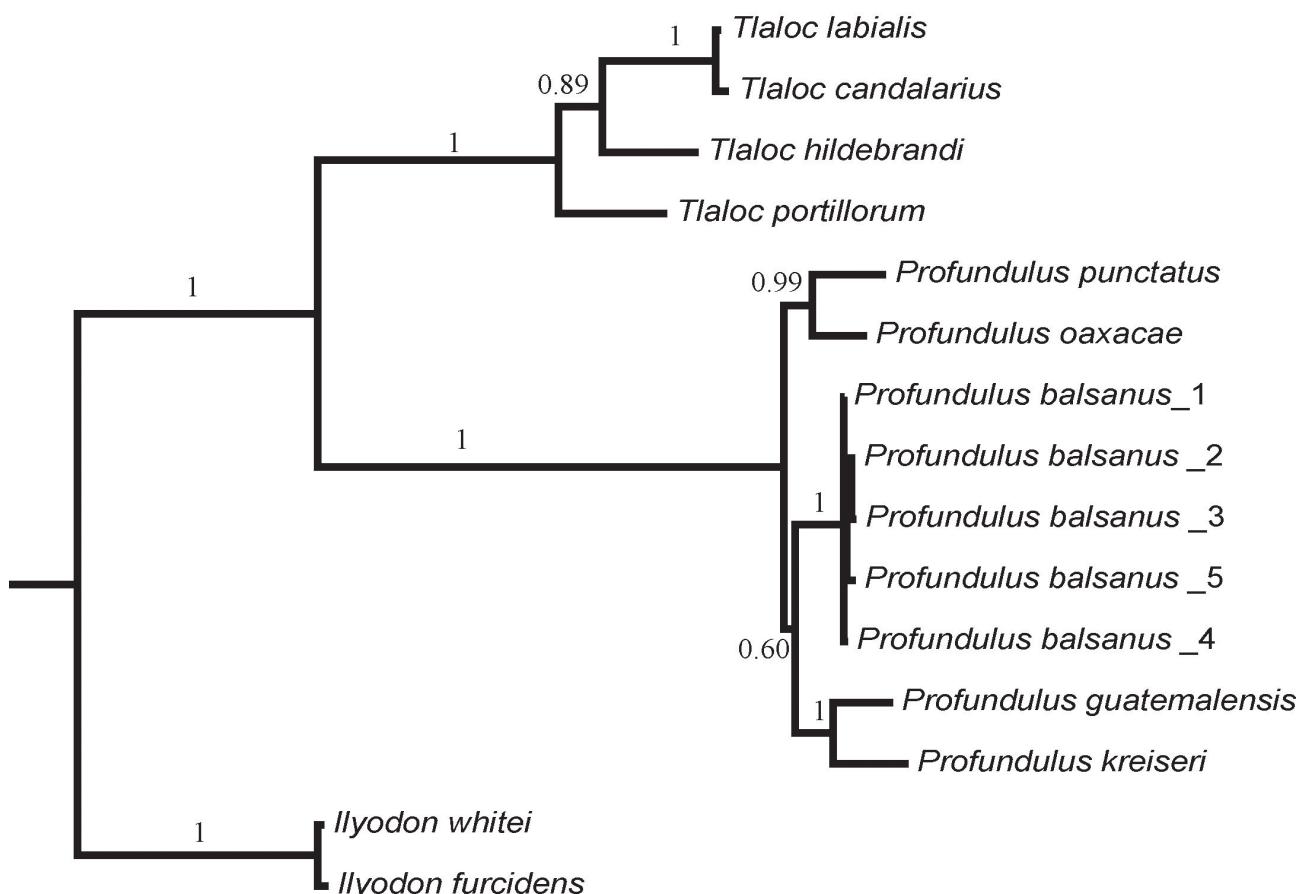
4b. Lower jaw not broad and heavy but like upper jaw and equal in forward projection or slightly protruding past upper jaw; body deep, no dark spot at caudal base ..... 5

5a. Lateral line scales typically 36–38 (34–39); vertebrae usually 36–37 (35–39); with predominantly six scales from midline of back to (but not including) scale in lateral series ..... *Tlaloc labialis*

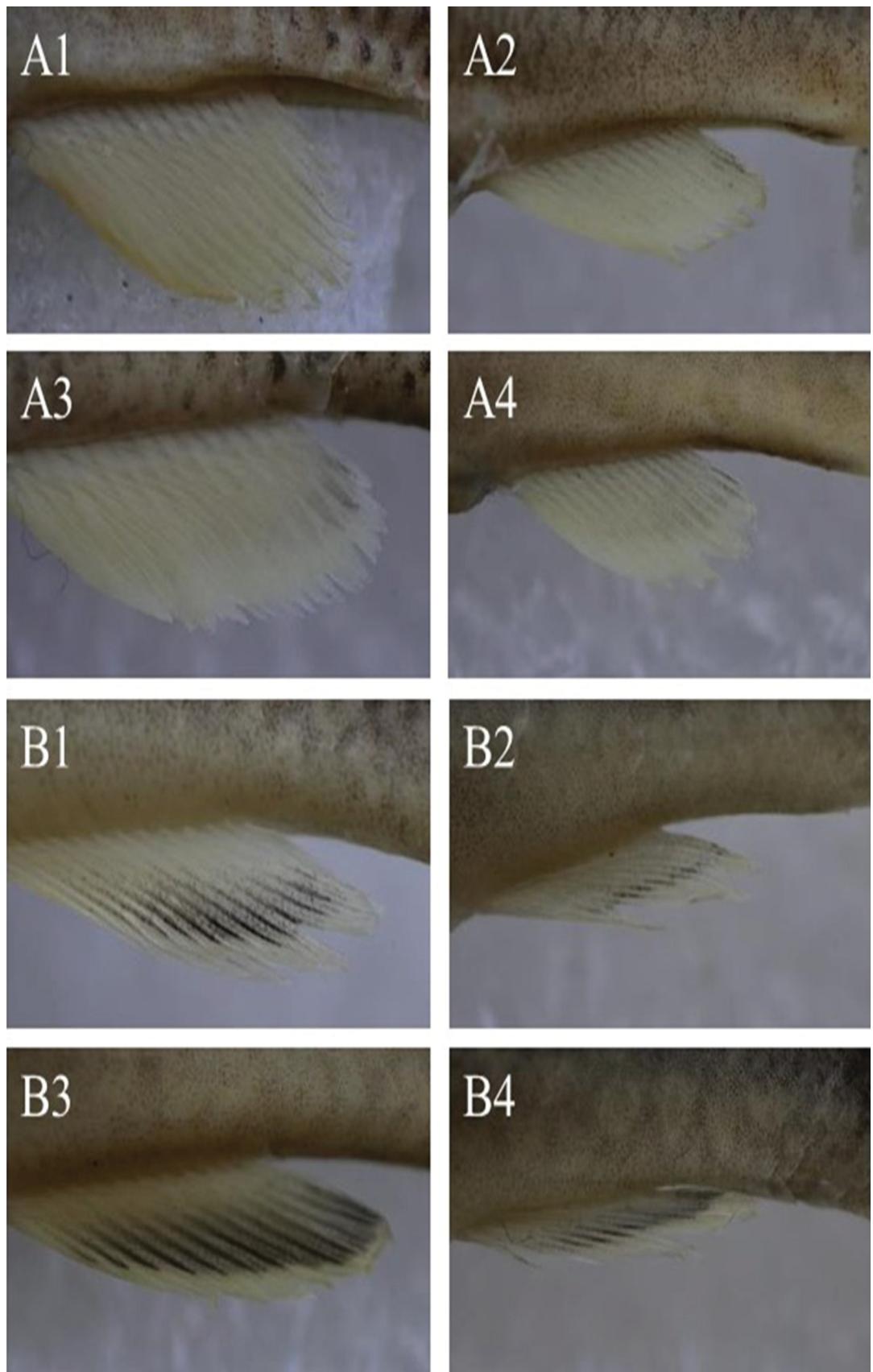
5b. Lateral-line scales usually 34 (33–36); vertebrae usually 34 (33–35); five scales from midline of back to (but not including) scale in lateral series ..... *Tlaloc candalarius*

## Acknowledgements

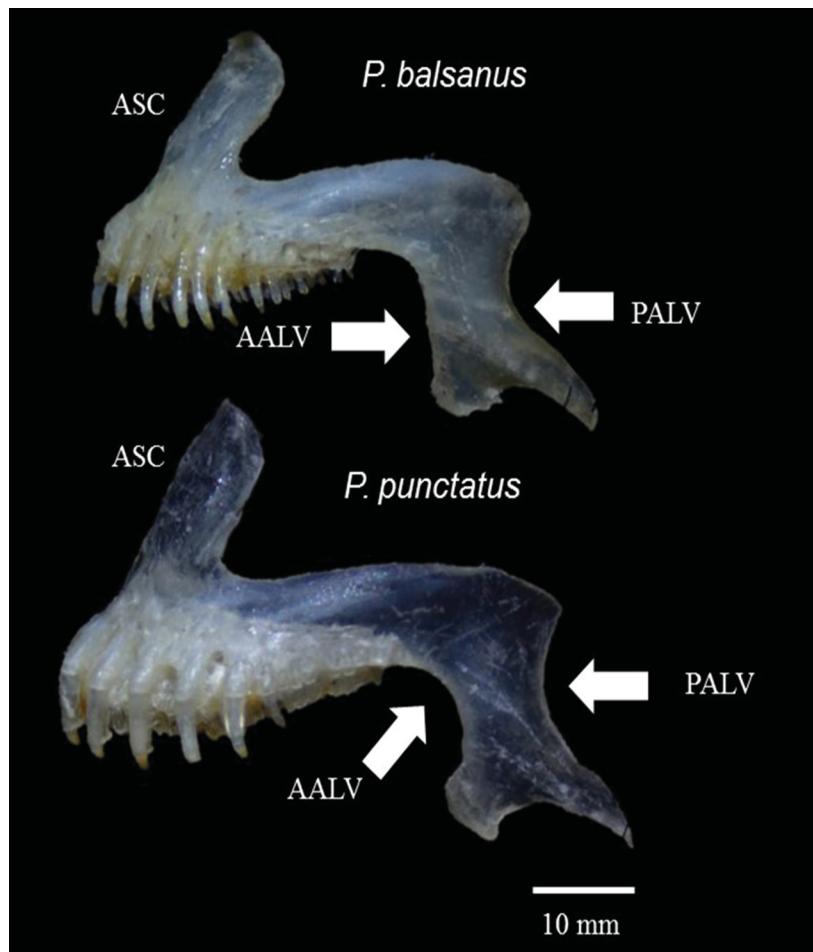
We thank Jorge Luis Lievano for preparation of the map and to all members of the UNICACH Ichthyology lab for their assistance with field and lab work. We thank Dr. Peter Bartsch from Museum of Natural History of Berlin for providing a photograph of the lectotype.



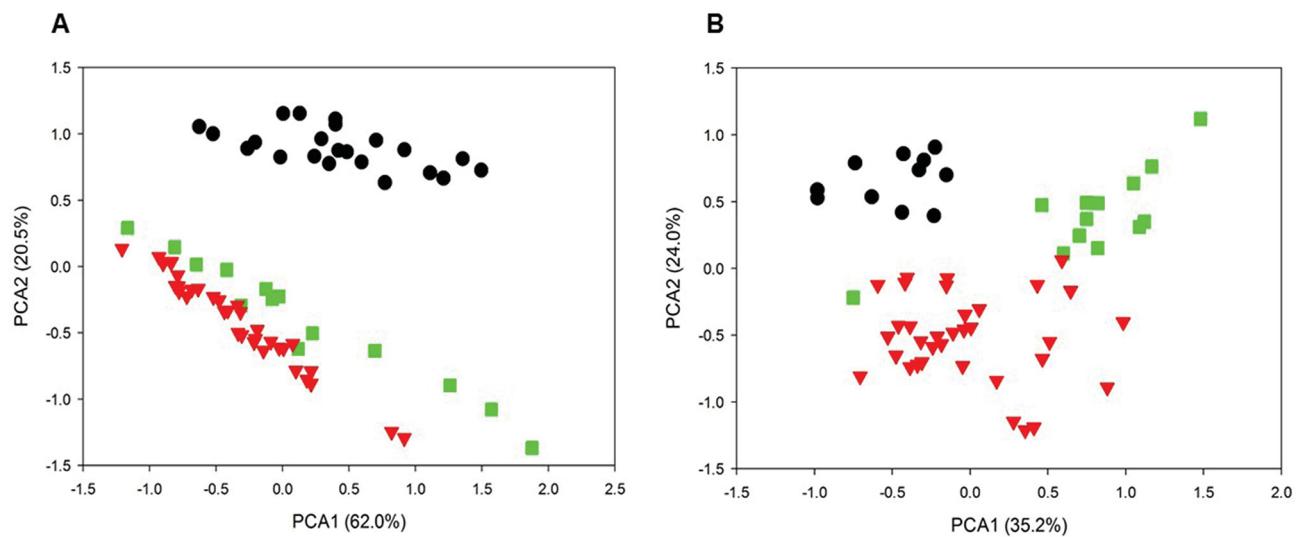
**FIGURE 4.** Bayesian phylogeny (based on concatenated Cyt-b and COI sequences) showing relationship of *Profundulus balsanus* with other members of the family Profundulidae. Numbers above nodes are Bayesian posterior probabilities.



**FIGURE 5.** Anal fins of *Profundulus balsanus* specimens (06712-MZ-P-UNICACH): (A1) adult female, (A2) juvenile female, (A3) adult male and (A4) juvenile male, showing a transparent anal-fin. Anal fins of *Profundulus punctatus* (06355-MZ-P-UNICACH): adult female (B1), juvenile female (B2), adult male (B3) and juvenile male (B4), showing an anal fin with a continuous transverse dark band in adults and less intense but continuous in juveniles.



**FIGURE 6.** Premaxilla of *P. punctatus* and *P. balsanus* showing the anterior alveolar process (AALV) and the Posterior Alveolar Process (PALV).



**FIGURE 7.** Results of Principal component analysis (PCA) for (A) body measurements and (B) meristic counts for *P. balsanus* (black circles), *P. punctatus* (green squares), and *P. oaxacae* (red triangles).

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## APPENDIX

**Material examined.** *Profundulus balsanus*, 06712-MZ-P-UNICACH, Río Malinaltepec, Malinaltepec, Guerrero, 535544°X, 1905700°Y, ACMJ-15-14, 10 of 46, 48.67–64.68 mm SL; 06574-MZ-P-UNICACH. Comparative material; *Profundulus punctatus*, 06355-MZ-P-UNICACH, Rio Zapata-Puente Col. Emiliano Zapata, Cacahoatán, Chiapas, 588614°X, 1657606°Y, AEGG-15-31, 14 of 18, 29.73–78.33 mm SL; *Profundulus oaxacae*, 06714-MZ-P-UNICACH, Río Salado-Subcuenca del Atoyac, Mitla, Oaxaca, 783936°X, 1873360°Y, ACMJ-15-16, 34 of 46, 26.46–63.19 mm SL.